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Theileria annulata and *T. parva* infect and transform different bovine mononuclear cells

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SUMMARY

Bovine peripheral blood mononuclear cells (PBMC) were labelled with monoclonal antibodies recognizing bovine MHC class II, sIgM, monocyte, T-helper and T-cytotoxic cell phenotypes. They were sorted into positive and negative populations with a fluorescence-activated cell sorter (FACS). The cell populations were infected *in vitro* with sporozoites of either *Theileria annulata* or *T. parva*, and the degree of infection and transformation determined. The results showed that despite the many similarities between these two parasites, they infected different cells of the immune system. *T. annulata* preferentially infected MHC class II-positive cells but did not infect T cells. Monocytes were infected very efficiently by *T. annulata* but were uninfected by *T. parva*. B cells were infected much more efficiently by *T. annulata* than *T. parva*. Cell lines derived from infections with *T. annulata* were analysed phenotypically. Virtually all reactivity was lost for the anti-sIgM and the anti-monocyte monoclonal antibodies post-infection and no T-cell markers were detected.

INTRODUCTION

Tropical theileriosis is a disease of major importance throughout North Africa and Southern Asia. It is caused by the protozoan parasite *Theileria annulata*, which is transmitted to the bovine host by ticks of the genus *Hyalomma*. East Coast fever, which occurs in East and Central Africa, is caused by the related parasite *T. parva* and is transmitted only by the tick *Rhipicephalus appendiculatus*. In addition to the differences between these two parasites in their geographical distribution and vector ticks, there are also differences in pathogenesis: *T. parva* produces greater mortality than *T. annulata*. However, a feature unique to both parasites is their ability to infect and transform cells of the immune system both *in vivo* and *in vitro* to produce continuously growing lymphoblastoid cell lines.

Although there have been several studies to identify the cell types infected and transformed by *T. parva*, it is not known which cells are infected with *T. annulata*. Cell lines arising from infection with *T. parva* generally show markers characteristic of T cells and most express MHC class II antigens but not sIgM (Naessens *et al.*, 1985; Lalor, Morrison & Black, 1986; Baldwin *et al.*, 1988). We have shown recently that established *T. annulata*-infected lines never express T-cell markers or surface (s)IgM but do express major histocompatibility complex (MHC) class II antigens (Spooner *et al.*, 1988).

It has been shown that *T. parva* infects T cells, B cells and null cells (Lalor *et al.*, 1986; Morrison *et al.*, 1986; Baldwin *et al.*, 1988). It is not established with certainty whether *T. parva* infects macrophages. One report suggested that macrophages cultured for several days could be infected (Moulton *et al.*, 1984), whereas others found no evidence of infection and transformation of fresh monocyte/macrophage populations (Morrison *et al.*, 1986; Baldwin *et al.*, 1988). In contrast preliminary evidence suggested that *T. annulata* infects adherent cells (Musiime, 1983). It is not known whether *T. annulata* infects and transforms T cells. The above evidence suggests that the two parasites may infect different cells of the immune system, which may have relevance to the observed differences between the two diseases.

In this paper we report infection of purified subpopulations of bovine peripheral blood mononuclear cells (PBMC), including monocytes, B cells and T cells, with either *T. annulata* or *T. parva* sporozoites. The results show that the two parasites infect and transform different cells types.

MATERIALS AND METHODS

Cell preparation

Blood was collected aseptically in 20% v/v acid citrate dextrose (ACD) from Friesian cows. Peripheral blood mononuclear cells (PBMC) were separated on Ficoll-Hypaque (Lymphoprep, NYCOMED AS, Oslo, Norway). Viability was always greater than 95% as assessed using Trypan blue dye exclusion.

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Sporozoite preparation

Hyalomma a. anatolicum ticks, previously infected with *T. annulata* Hissar or Ankara (Walker *et al.*, 1985) and fed for 3 days on rabbits, were ground up in MEM (Gibco, Paisley, Renfrewshire) with 3.5% bovine plasma albumin (BPA) (Sigma, Poole, Dorset). The supernatant was filtered and harvested as ground up tick supernatant (GUTS). The *T. parva* sporozoites were prepared from previously infected *Rhipicephalus appendiculatus* ticks fed for 4 days on rabbits. Salivary glands were dissected into Liebowitz L15 (Gibco) and homogenized and subsequently diluted in MEM + 3.5% BPA. (Brown, 1987). The *T. annulata* GUTS filtrates were used at 0.5 tick equivalents/ml and the *T. parva* salivary gland homogenate at 10 tick equivalents/ml. This produced approximately equal levels of infectivity for the two parasites on unsorted PBMC. Each experiment utilized a fresh batch of ticks.

Monoclonal antibodies

Five monoclonal antibodies (mAbs) were used in this study; IL-A11 detects BoT4, the bovine equivalent of human T4 (Baldwin *et al.*, 1986; Teale *et al.*, 1986), IL-A17 detects BoT8, the bovine equivalent of human T8 (Ellis *et al.*, 1986), B5/4 reacts with bovine sIgM (Pinder *et al.*, 1980) and IL-A24 reacts with monocytes and granulocytes (Ellis *et al.*, 1987; Glass & Spooner, 1989). J11 reacts with bovine MHC class II (Baldwin, Morrison & Naesens, 1987). The mAbs used for preparing cells for FACS sorting were diluted to 1:100 in RPMI-1640 with 25 mM HEPES and 10% fetal calf serum (FCS) (Gibco), dialysed against RPMI-1640 with 25 mM HEPES for 24 hr, and filter sterilized.

Immunofluorescent staining

For analysis. This was performed as described by Spooner *et al.* (1988).

For FACS sorting. Indirect membrane staining was performed in RPMI-1640 with 25 mM HEPES and 5% gamma globulin-free horse serum (HS) (Gibco). Two millilitres PBMC at 10^7 cells/ml were mixed with an equal volume of diluted mAb at 4° . The mAbs were used at dilutions which gave maximal staining. After 30 min incubation at 4° the cells were washed three times in RPMI-1640 containing 25 mM HEPES, 5% HS, 100 IU/ml penicillin and 100 μ g/ml streptomycin (sorting medium) and then 1 ml of a FITC-conjugated anti-mouse Ig (RAM Ig; Nordic, Maidenhead, Somerset) was added at a dilution of 1:40. After 30 min incubation at 4° the cells were again washed three times in sorting medium and maintained at 4° prior to sorting.

Cell sorting

Cells were sorted on a FACS IV fluorescence-activated cell sorter (Becton-Dickinson & Co., FACS Systems, Sunnyvale, CA). The calibration of the equipment was as described by Spooner *et al.* (1988). Cells were collected in sterile phosphate-buffered saline pH 7.2 (PBS) and samples of the negative and positive populations were re-analysed to estimate the efficiency of the separation. The sorted cells were washed once at 100 g and resuspended in RPMI-1640 with 25 mM HEPES, 20% FCS, 200 IU/ml penicillin and 200 μ g/ml streptomycin.

Cell infections

Fixed concentration (T. annulata only). Subpopulations of PBMC enriched or depleted for T cells were produced by FAC sorting using a combination of mAbs IL-A11 (T4) and IL-A17

(T8). Populations depleted or enriched for MHC class II-positive cells were produced using mAb J11. The cells were adjusted to 2×10^6 cells/ml. Equal volumes (100 μ l of cell suspension and 100 μ l *T. annulata* sporozoite suspension) were incubated together in triplicate in 96-well flat-bottomed tissue culture plates in a 5% CO₂/air mixture at 38° . At 4 days post-infection, cytospin samples were prepared (see below) and the percentage of infected cells estimated for each subpopulation.

Limiting dilutions (T. annulata and T. parva separately). Sorted cells at 10^6 /ml or lower, depending on availability, were mixed with equal volumes of the sporozoite preparation and incubated at 38° for 1 hr. Log₃ dilutions of this mixture were then made in complete tissue culture medium (RPMI-1640 with 25 mM HEPES, 10% FCS, 2 mM L-glutamine with 100 IU/ml penicillin and 100 μ g/ml streptomycin). Then 20 replicate cultures of 200 μ l/well were set up in 96-well (round-bottomed) culture plates in log₃ dilution steps to 1 cell/well. In some cases, such as with the IL-A24-positive sort, fewer cells were available and the highest concentration was 10^2 cells/well. Filler cells (PBMC exposed to 5000 rads of gamma irradiation) were added to each well for the *T. parva* infections at 5×10^4 cells/well. No filler cells were used for the *T. annulata* infections. Transformation was assessed by the presence of blast cells at 4 days, using a Leitz Diavert phase-contrast microscope (Leitz, Wetzlar, FRG). Transformed cells were at least four times larger than untransformed cells and could be seen easily without staining. The plates were then maintained in 5% CO₂/air mixture for a further 3 weeks. There was no significant change in the dilution to which transformation was seen between 4 days and 3 weeks. Thus only the Day 4 readings were used.

Transformed cells were removed from individual wells in the limiting dilution plates at 19 days post-infection. These were stained with mAbs and then analysed with the FACS as described by Spooner *et al.* (1988).

Cytospins

Cytopentrifuge smears (Cytospin, Shandon, Runcorn, Cheshire) were prepared from representative wells from the limiting-dilution experiments and flat wells. These were stained with Giemsa and examined with an Ortholux II microscope at $1250\times$ magnification (LEITZ). The percentage of parasite-infected cells was estimated from counting 200 cells.

RESULTS

Infections with *T. annulata*

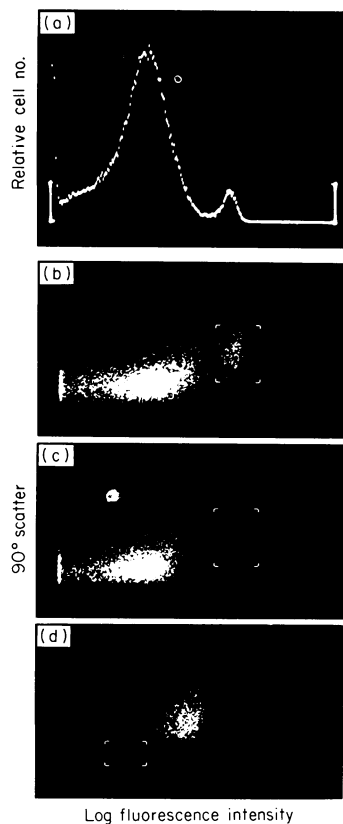
To determine which cell populations of PBMC could be infected with *T. annulata* sporozoites, subpopulations of PBMC were isolated using the FACS. T cells, non-T cells, MHC class II-positive and class II-negative cells were incubated with *T. annulata* sporozoites and the percentage of transformed infected cells was assessed. The results of two experiments with cells from different animals on different days are shown in Table 1. Here it can be seen that *T. annulata* infects class II-positive cells very efficiently and T cells very inefficiently.

Infections with *T. annulata* and *T. parva*

The results of infection with *T. annulata* only showed that this parasite infected and transformed class II-positive cells very

Table 1. Infection of mononuclear cell subpopulations with *T. annulata*

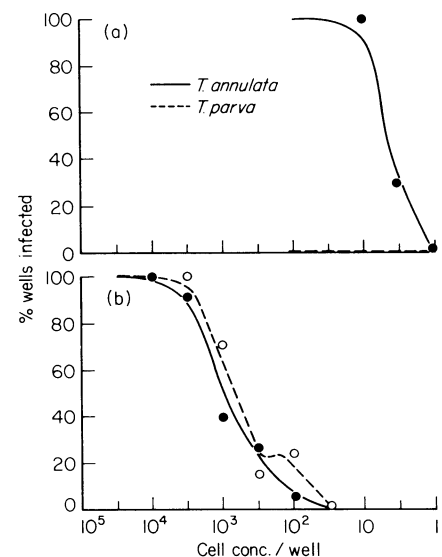
Animal providing PBMC:	Infected cells at 4 days	
	Animal 1	Animal 2
Subpopulation		
T-cell enriched	8	< 10
T-cell depleted	75	100
MHC class II positive	81	90
MHC class II negative	10	< 1

**Figure 1.** FACS profiles of PBMC stained with IL-A24 (a) showing a large negative and small (5%) positive population. (b), (c) and (d) show the scatter plots before and after sorting. The sort windows are shown and there is negligible contamination in the two sorted samples. The IL-A24-positive cells have a higher 90° scatter profile as expected with a monocyte population.

efficiently. To study this further, the two main cellular components of the MHC II-positive cells in peripheral blood, namely monocytes and B cells, were prepared and aliquots were infected with either *T. annulata* or *T. parva* sporozoites.

Monocytes

PBMC were sorted on the FACS using the monocyte-specific mAb IL-A24. The initial percentage of IL-A24-positive cells in PBMC varied between 4% and 6% on different days (data not shown). The FACS profiles of the unsorted and positive and

**Figure 2.** Infection and transformation with *T. annulata* and *T. parva*, (a) IL-A24-positive cells where the maximum cell concentration available was 100 cells/well, and (b) IL-A24-negative cells.**Table 2.** FACS analysis of cell lines arising from infection of PBMC and sorted cells with *T. annulata* showing the percentage of cells positive for MHC class II, T4, T8, monocyte and sIgM antigens 19 days post-infection

Antigen detected	PBMC pre-infection	Cells from which lines derived				
		PBMC	IgM		IL-A24	
			+	—	+	—
Class II	20.2	95.7	82.6	85.8	43.6	96.5
T4	30.0	0.9	4.5	3.5	1.6	1.0
T8	33.7	0.7	5.9	2.4	1.5	1.1
Monocytes	4.0	2.3	2.9	4.6	9.8	2.1
IgM	23.0	1.1	6.2	4.3	1.3	2.7

negative populations are shown in Fig. 1. The IL-A24-positive sample was contaminated with 1% negative cells and the negative sample with 4% positive cells. These sorted populations were then incubated with sporozoites of either *T. parva* or *T. annulata* and the degree of infection and transformation estimated. The *T. annulata* infection curve for the monocyte preparation (Fig. 2a) indicates that almost every IL-A24-positive cell was infected and transformed. In contrast *T. parva* did not infect any IL-A24-positive cells.

With the IL-A24-negative population (Fig. 2b) there was no difference in infectivity between *T. annulata* and *T. parva* sporozoites. In fact the infectivity of this population was indistinguishable from that of unsorted PBMC (data not shown).

B cells

The anti-sIgM mAb, B5/4, was used to prepare a purified population of B cells from PBMC. The proportion of sIgM-positive cells in the initial PBMC sample was 23% (Table 2).

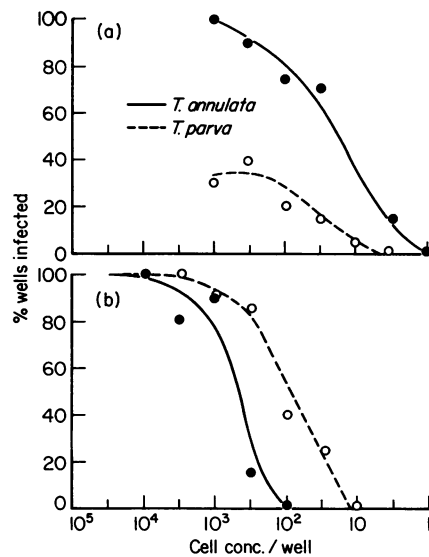


Figure 3. Infection and transformation by *T. annulata* and *T. parva* of (a) sIgM-positive and (b) sIgM-negative cells.

Figure 3a shows that essentially all sIgM-positive cells were infected by *T. annulata*, whereas infection by *T. parva* was less efficient and never more than 30% of the wells were infected at any cell concentration. Figure 3b shows that *T. parva* infected sIgM-negative cells more efficiently (10-fold) than *T. annulata*.

Phenotypic analysis

Cells from individual wells from the limiting-dilution plates of the *T. annulata* infections were analysed phenotypically by indirect immunofluorescence and the results are shown in Table 2. Cell phenotypes arising from transformation of PBMC, sIgM and IL-A24-positive and negative populations are indistinguishable using the available mAbs after 19 days post-infection. All the lines were MHC class II positive, though to varying degrees. T cell, monocyte and sIgM markers were either not expressed or only expressed at very low levels. We did not analyse transformed cells arising from the *T. parva* infections as they were contaminated with filler cells and long-term lines were not created.

DISCUSSION

We have shown for the first time that *T. annulata* and *T. parva* infect different cells of the bovine immune system. Virtually 100% of monocytes and B cells are infected with *T. annulata*, whereas T cells are not infected. In contrast *T. parva* does not infect monocytes and infects B cells less efficiently than *T. annulata*. The evidence presented in this paper generally supports other recent studies on the cell types infected by *T. parva* (Baldwin *et al.*, 1988). However, our results indicate that either *T. parva* infects B cells inefficiently or only infects a subpopulation. This would fit with the findings of Baldwin *et al.* (1988) that it is difficult to produce sIgM-positive lines following *T. parva* infection unless the cells are cloned immediately after infection.

All of the cell lines arising from infection with *T. annulata* have the same phenotype, regardless of the cell subpopulation

infected. They are all MHC class II positive but lack all reactivity for T-cell markers, and by 19 days post-infection have very low levels of monocyte and B-cell markers. This agrees with data on 34 long-term *T. annulata*-infected cell lines, none of which expressed T-cell, B-cell or monocyte markers but were all class II positive (Spooner *et al.*, 1988). This indicates that although the sporozoites clearly infected both monocytes and B cells, the IL-A24 marker and sIgM are rapidly down-regulated. Current work is in progress to determine how and when this occurs.

The findings reported here raise a number of important questions with regard to infection with *T. annulata* and *T. parva*. If these *in vitro* results reflect the situation *in vivo*, there are several possible consequences relating to the pathogenesis of the diseases. The cell types infected by *T. annulata* already express class II antigens and maintain them despite infection. Therefore there is no a priori reason why presentation of *T. annulata* antigens to the immune system should not occur immediately. However, the cell types infected by *T. parva* do not generally express class II molecules. These would have to be switched on before infected cells could be recognized by T helper (Th) cells. Thus the initiation of an immunological response to *T. parva* may be slower than that with *T. annulata*. This may help to explain why there is higher morbidity and mortality with *T. parva* than with *T. annulata*.

It has been shown that an important effector mechanism during both *T. parva* (Morrison *et al.*, 1988) and *T. annulata* (Innes *et al.*, 1989a) infection involves class I-restricted T cytotoxic (Tc) cells. Moreover, cell lines infected with *T. annulata* or *T. parva* both express MHC class I antigens to a similar degree (Spooner & Brown, 1980). Thus the differences between the two diseases would not appear to be explained by differences in CTL function.

The fact that *T. annulata* infects antigen-presenting cells (APC) whereas *T. parva* infects non-APC may lead to differences in the functioning of the immune system and may affect not only the response to the diseases themselves but also the response to intercurrent infections. Infection of APC by *T. annulata* may suppress their ability to present third-party antigens. The initial characterization of the mAb IL-A24 suggests that the molecule that it recognizes is involved in antigen presentation (Ellis *et al.*, 1987) and more recently we have demonstrated the antigen-presenting cell function in cattle is associated with IL-A24-positive, MHC class II-positive adherent cells (Glass & Spooner, 1989). The down-regulation of this antigen may adversely affect APC function. Conversely *T. parva* infection of T cells may suppress the ability of Th cells to help or Tc cells to kill. However, an alloreactive cytotoxic T-cell line did maintain cytotoxic function for a limited period after infection with *T. parva* (Baldwin & Teale, 1987). We are currently investigating these possibilities.

There are further differences between the two parasites. With *T. annulata*, cell lines generated both *in vivo* (Pipano, 1981) and *in vitro* (Ouhelli *et al.*, 1989) can be used to immunize genetically unrelated cattle. By contrast, with *T. parva* only high doses of allogeneic infected cells will immunize cattle (Brown, 1981). We have shown that at low cell doses cattle are only immunized against *T. parva* if the recipient animal and the immunizing cell line have the same major histocompatibility complex (MHC) class I BoLA type (Teale, 1983; Dolan *et al.*, 1984). This histocompatibility barrier to immunization does not

occur with *T. annulata*, even with as few as 100 allogeneic cells (Innes *et al.*, 1989b). It is not clear how the different cell preferences of the two parasites could affect the ease of immunization. If schizonts (the intracellular stage of the parasite) have the same cellular preferences *in vivo* as we have shown for sporozoites *in vitro* this might help to explain why it is easier to infect and immunize animals with *T. annulata* than with *T. parva*.

T. annulata and *T. parva* thus provide a unique model for dissecting the bovine immune system that may have wider relevance to immunological questions in other species.

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REFERENCES

- BALDWIN C.L., BLACK S.J., BROWN W.C., CONRAD P.A., GODDEERIS B.M., KINUTHIA S.W. *et al.*, (1988) Bovine T-cells, B-cells and null cells are transformed *in vitro* and *in vivo* by the protozoan parasite *Theileria parva*. *Infect. Immun.* **56**, 462.
- BALDWIN C.L., MORRISON W.I. & NAESSENS J. (1987) Differentiation antigens and functional characteristics expressed by bovine leukocytes. In: *Differentiation Antigens in Lympho-Haemopoietic Tissues* (eds M. Miyasaka and Z. Trnka), p. 455. Marcel Dekker, New York & Basel.
- BALDWIN C.L. & TEALE A.J. (1987) Alloreactive T cell clones transformed by *Theileria parva* retain cytotoxic activity and antigen specificity. *Eur. J. Immunol.* **17**, 1859.
- BALDWIN C.L., TEALE A.J., NAESSENS J.G., GODDEERIS B.M., MACHUGH N.D. & MORRISON W.I. (1986) Characterization of a subset of bovine T lymphocytes that express BoT4 by monoclonal antibodies and function: similarity to lymphocytes defined by human T4 and murine L3T4. *J. Immunol.* **136**, 4385.
- BROWN C.G.D. (1981) Application of *in vitro* techniques to vaccination against theileriosis. In: *Advances in the Control of Theileriosis* (eds A. D. Irvin, M. P. Cunningham and A. S. Young), Current Topics in Veterinary Medicine & Animal Science Vol. 14, p. 104. Martinus Nijhoff, The Hague.
- BROWN C.G.D. (1987) Theileriidae. In: *In-vitro Methods of Parasite Cultivation* (eds A. E. R. Taylor and J. R. Baker), pp. 230–253. Academic Press, London.
- DOLAN T.T., TEALE A.J., STAGG D.A., KEMP S.J., COWAN K.M., YOUNG A.S., GROOCKOCK C.M., LEITCH B.L., SPOONER R.L. & BROWN C.G.D. (1984) A histocompatibility barrier to immunization against East Coast fever using *Theileria parva*-infected lymphoblastoid cell lines. *Parasite Immunol.* **6**, 243.
- ELLIS J.A., BALDWIN C.L., MACHUGH N.D., BENSARD A., TEALE A.J., GODDEERIS B.M. & MORRISON W.I. (1986) Characterization by a monoclonal antibody and functional analysis of a subset of bovine T lymphocytes that express BoT8, a molecule analogous to human CD8. *Immunology*, **58**, 351.
- ELLIS J.A., MORRISON W.I., GODDEERIS B.M. & EMERY D.L. (1987) Bovine mononuclear phagocytic cells: identification by monoclonal antibodies and analysis of functional properties. *Vet. Immunol. Immunopathol.* **17**, 125.
- GLASS E.J. & SPOONER R.L. (1989) Requirement for MHC class II positive accessory cells in an antigen specific bovine T cell response. *Research Vet. Sci.* (in press).
- INNES E.A., MILLAR P., BROWN C.G.D. & SPOONER R.L. (1989a) The development and specificity of cytotoxic cells in cattle immunized with autologous or allogeneic *Theileria annulata* infected lymphoblastoid cell lines. *Parasite Immunol.* (in press).
- INNES E.A., OUHELLI H., OLIVER R.A., SIMPSON S.P., BROWN C.G.D. & SPOONER R.L. (1989b) The effect of MHC compatibility between parasite infected cell line and recipient in immunisation against tropical theileriosis. *Parasite Immunol.* (in press).
- LALOR P.A., MORRISON W.I. & BLACK S.J. (1986) Monoclonal antibodies to bovine leucocytes define heterogeneity of target cells for *in vitro* parasitosis by *Theileria parva*. In: *The Ruminant Immune System in Health and Disease* (ed. W. I. Morrison), pp. 72–87. Cambridge University Press.
- MORRISON W.I., LALOR P.A., GODDEERIS B.M. & TEALE A.J. (1986) Theileriosis: antigens and host-parasite interactions. In: *Parasite Antigens: Toward new Strategies for Vaccines* (ed. T. W. Pearson), p. 167. Marcel Dekker, New York.
- MOULTON J., BUSCHER G., BOVELL D. & DOXSEY S. (1984) Blast transformation of adherent macrophages infected *in vitro* with sporozoites of *Theileria parva*. *Amer. J. Vet. Res.* **45**, 678.
- MUSIIME J.T. (1983) *In vitro studies of immune mechanisms in bovine theileriosis*. Ph.D. Thesis, University of Edinburgh.
- NAESSENS J., NEWSON J., BENSARD A., TEALE A.J., MAGONDU J.G. & BLACK S.J. (1985) *De novo* expression of T cell markers on *Theileria parva*-transformed lymphoblasts in cattle. *J. Immunol.* **135**, 4183.
- OUHELLI I.H., INNES E.A., BROWN C.G.D., WALKER A.R., SIMPSON S.P. & SPOONER R.L. (1989) The effect of cell dose and cell line on immunisation of cattle with *Theileria annulata* infected lymphoblastoid cell lines. *Vet. Parasitol.* (in press).
- PINDER M., MUSOKE A.J., MORRISON W.I. & ROELANTS G.E. (1980) The bovine lymphoid system. III. A monoclonal antibody specific for bovine cell surface and serum SIgM. *Immunology*, **40**, 359.
- PIPANO E. (1981) Schizonts and tick stages in immunization against *Theileria annulata* infection. In: *Advances in the Control of Theileriosis* (eds A. D. Irvin, M. P. Cunningham and A. S. Young), Current Topics in Veterinary medicine & Animal Science, Vol. 14, p. 242. Martinus Nijhoff, The Hague.
- SPOONER R.L. & BROWN C.G.D. (1980) Bovine lymphocyte antigens (BoLA) of bovine lymphocytes and derived lymphoblastoid lines transformed by *Theileria parva* and *Theileria annulata*. *Parasite Immunol.* **2**, 163.
- SPOONER R.L., INNES E.A., GLASS E.J., MILLAR P. & BROWN C.G.D. (1988) Bovine mononuclear cell lines transformed by *Theileria parva* or *Theileria annulata* express different subpopulation markers. *Parasite Immunol.* **10**, 619.
- TEALE A.J. (1983) *The major histocompatibility complex of cattle with particular reference to some aspects of East Coast fever*. Ph.D. Thesis, University of Edinburgh.
- TEALE A.J., BALDWIN C.L., ELLIS J.A., NEWSON J., GODDEERIS B.M. & MORRISON W.I. (1986) Alloreactive bovine T lymphocyte clones: an analysis of function, phenotype, and specificity. *J. Immunol.* **136**, 4392.
- WALKER A.R., FLETCHER J.D., MCKELLAR S.B., BELL L.J. & BROWN C.G.D. (1985) The maintenance and survival of *Theileria annulata* in colonies of *Hyalomma anatolicum anastolicum*. *Ann. Trop. Med. Parasitol.* **79**, 199.